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13. ABSTRACT (Maximum 200 Words)

The purpose of this proposal is to evaluate the role of IQGAP1 in neoplastic transformation and metastasis of breast epithelial cells. The main emphasis is on whether IQGAP1 is involved in invasion and metastasis of transformed breast epithelial cells, as well as the possible involvement of IQGAP1 in regulating β-catenin function. Major findings to date are: (i)there is a high level of expression of IQGAP1 in breast epithelium; (ii)overexpression of IQGAP1 in mammalian cells enhances cell migration and proliferation; and (iii) decreased IQGAP1 protein levels reduced cell motility. These data reveal that IQGAP1 has a fundamental role in cell motility and invasion in breat epithelium. This information could have potential therapeutic implications in patients with breast cancer.

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INTRODUCTION

Malignancy results from altered modulation of cell adhesion and motility. IQGAP1 is a critical link in the signal transduction pathways leading to enhancement of cell motility and altered cell-cell adhesion. IQGAP1 appears to act as a focal point of cross-talk where diverse regulatory and structural proteins converge. Importantly, IQGAP1 is significantly increased in highly metastatic melanoma and gastric cells. The hypothesis to be evaluated in this proposal is that elevated levels of IQGAP1 promote neoplastic transformation and metastasis. The role of IQGAP1 in cell motility and proliferation has been examined. In addition, IQGAP1 also interacts with β-catenin, a proto-oncogene that participates in cell-cell adhesion and transcriptional co-activation. The effects of IQGAP1 on the stability, subcellular localization and transcriptional co-activation of β-catenin will be assessed. Elucidation of the role played by IQGAP1 in promoting neoplasia and metastasis will enhance our comprehension of the molecular mechanisms responsible for breast cancer.

BODY

Research accomplishments are described according to the Tasks listed in the approved Statement of Work. These results encompass the period 1st June 2003 to 31st May 2004.

Task 1. Ascertain whether IQGAP1 is involved in invasion and metastasis of transformed cells

i. Examine IQGAP1 protein levels in cell types of varying malignancy

Our studies involving analysis of selected cell lines revealed that there is a very high level of expression of IQGAP1 in breast epithelium. Experiments to examine the relative levels of expression of IQGAP1 protein in multiple human breast carcinoma cell lines – MCF-7, ZR-75-1, T-47D, MDA-MB-231, 21T and Hs578T – and non-tumorigenic MCF-10 and MTSV-1 are ongoing. In addition, our collaborator, Richard Hynes at the Center for Cancer Research at MIT observed that IQGAP1 concentrations in metastatic melanoma cells are higher than those in non metastatic cells. These findings support the hypothesis that highly metastatic cells express increased IQGAP1 protein.

ii. Determine if IQGAP1 overexpression promotes tumour cell migration

Migration of MCF-7 cells stably transfected with pcDNA3 vector (termed MCF/V cells) and MCF-7 cells stably transfected with pcDNA3-IQGAP1 (termed MCF/I cells) expressing IQGAP1 at three times the levels expressed in MCF/V cells was evaluated. As shown in our previous report (Annual report dated June 2003), MCF/I cells exhibited a 2.8 ± 0.17-fold (mean ± S.E., n=16, p<0.005) greater motility than MCF/V cells. Similarly, transient overexpression of IQGAP1 accelerated motility by

1.6 ± 0.07-fold (n=4, p<0.005) and 1.7 ± 0.09-fold (n=4, p<0.005) in HEK-293H cells and highly motile MDA-MB-231 cells, respectively. The role of IQGAP1 in increased cell motility implies that IQGAP1 could also contribute to cell invasion. For invasion assays, motility of cells through Matrigel-coated Transwells was monitored. Our results reveal that MCF/I cells were 2.5-fold more invasive than MCF/V cells.

iii. Determine if IQGAP1 overexpression promotes tumour cell proliferation

Studies have been initiated to address this task. As outlined in our previous report (June 2003) equal numbers of MCF/V and MCF/I cells were examined for their [^{3}H]thymidine uptake to measure DNA synthesis and therefore, cell proliferation. Initial analysis reveals that, compared to MCF/V cells, MCF/I cells increased [^{3}H]thymidine uptake (data not shown). Further analysis is scheduled to commence in the third year of funding.

iv. Develop antisense oligonucleotides to inhibit IQGAP1 expression and analyze the effect of decreased IQGAP1 protein levels on cell migration and proliferation

Originally, antisense oligonucleotides were proposed to inhibit IQGAP1 protein expression. However, after submission of the original proposal, small interfering RNA (siRNA) was developed. This technique is an efficient method to specifically knock down individual proteins in mammalian cells. Because RNA interference offers many advantages over antisense oligos adopted this approach to disrupt IQGAP1 function in cells. Several siRNA oligonucleotides were designed, targeting different regions of IQGAP1. Transient expression of a 19-mer oligonucleotide complementary to basepair (bp) 4959–4977 of IQGAP1 cDNA, termed siRNA 8, reduced IQGAP1 protein levels by over 50% (Fig.1A). The downregulation was specific for IQGAP1 as levels of Ras, Raf, MEK, ERK2, actin, Cdc42 and Rac1 (data not shown) were not altered. Importantly, reduction of IQGAP1 protein by siRNA 8 significantly retarded the ability of MCF-7 cells to migrate through Transwell pores (Fig. 1B). In order to verify the specificity of the siRNA, another oligonucleotide was utilized, termed siRNA 9, which is directed against bp 6705–6723 of IQGAP1. Transfection of siRNA 9 significantly reduced both endogenous IQGAP1 (Fig. 1A) and cell migration through Transwell pores (Fig. 1B). By contrast, siRNAs 2, 3, 5, and 6, which do not reduce IQGAP1 protein expression, had no effect on cell motility (Fig. 1A and B).

We employed a retroviral system to stably integrate siRNA 8 into the genome of MCF-7 cells. IQGAP1 protein expression in these cells (termed MCF-siIQ8 cells) was reduced by 80% (Fig. 2A). Compared with native MCF-7 cells, cell migration of MCF-siIQ8 cells was decreased by 71% (Fig. 2B). The magnitude of reduction of cell migration correlated with the extent of the decrease in IQGAP1 protein levels. These data strongly suggest that IQGAP1 is required for cell motility. Since cell motility is influenced by growth factors we looked at mitogen activated protein (MAP) kinase

signalling in MCF/V, MCF/I and MCF-siIQ8 cells by measuring epidermal growth factor (EGF)-stimulated changes in extracellular signal-regulated kinase (ERK)1/2 phosphorylation.

Incubation of serum-starved MCF/V cells with EGF enhanced ERK activity by 4.3 ± 0.27-fold (n=4) (Fig. 3). Overexpression of IQGAP1 (in MCF/I cells) reduced by 50% the ability of EGF to stimulate ERK phosphorylation. Remarkably, EGF was unable to activate ERKs in MCF-siIQ8 cells which have reduced endogenous IQGAP1 (Fig. 3). Our results suggest that an optimal level of intracellular IQGAP1 is required for maximal activation of ERK1 and ERK2 by EGF.

Task 2. Test the hypothesis that IQGAP1 regulates β-catenin function in breast cancer cell lines of varying malignancy

This task is scheduled to commence in the third year of funding.

KEY RESEARCH ACCOMPLISHMENTS

- there is a high level of expression of IQGAP1 in breast epithelium
- overexpression of IQGAP1 in mammalian cells enhances cell migration
- IQGAP1 overexpressing MCF-7 cells are more invasive than vector-transfected MCF-7 cells
- overexpression of IQGAP1 enhances cellular proliferation
- decreased IQGAP1 protein levels reduced cell motility

REPORTABLE OUTCOMES

None

CONCLUSIONS

The work performed to date has yielded some insights into the role of IQGAP1 in cell motility. We observed that there is a high level of IQGAP1 protein expressed in breast epithelium. In addition, overexpression of IQGAP1 in transformed breast cells enhanced cell migration and cell proliferation. In contrast, decreased IQGAP1 levels reduced cell motility. Initial findings support the hypothesis that IQGAP1 plays a significant role in the metastasis of breast epithelial cells. This information could help determine whether IQGAP1 is a potential therapeutic target for breast cancer.

REFERENCES

None

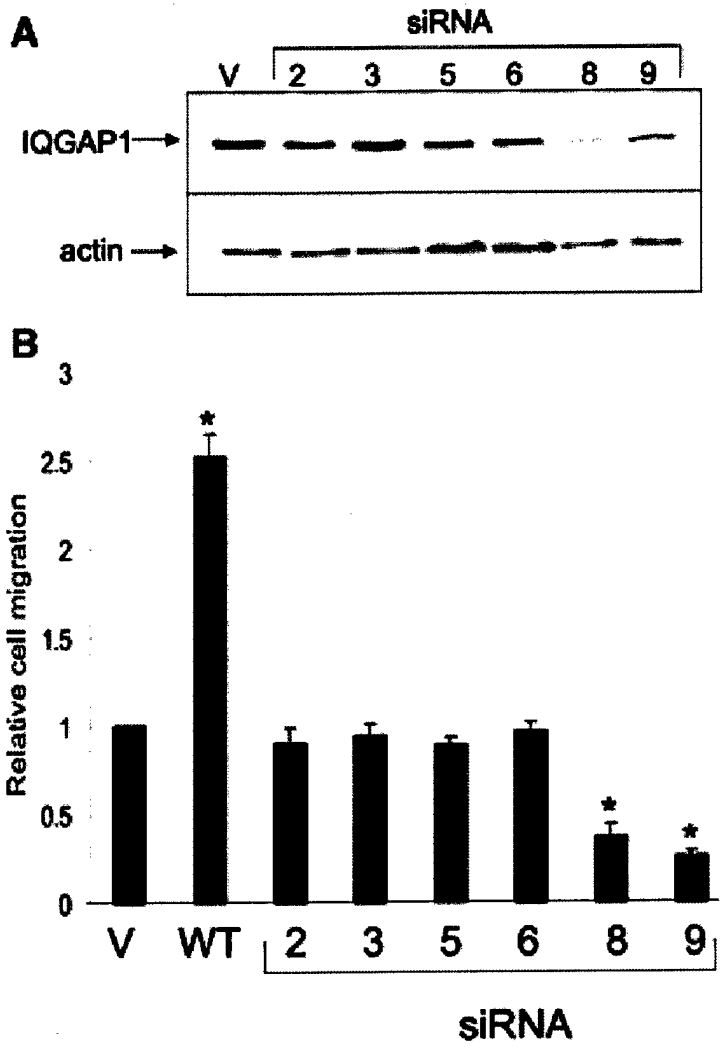

Fig. 1. Reduction of IQGAP1 by siRNA attenuates cell migration. *A*, MCF-7 cells were transiently transfected with mU6pro (*V*) or mU6siRNA 2, 3, 5, 6, 8, or 9. Equal amounts of protein lysates were analyzed by Western blotting with anti-IQGAP1 and anti-actin antibodies. *B*, MCF-7 cells were transfected with vector (*V*), IQGAP1 (*WT*), or mU6siRNA 2, 3, 5, 6, 8, or 9. Migration data, expressed relative to vector-transfected cells, represent the means ± S.E. (n = 6 for vector, wild type, and mU6siRNA 8, and n = 2 for mU6siRNA 2, 3, 5, 6, and 9). *, significantly different from vector-transfected cells ($p < 0.001$).

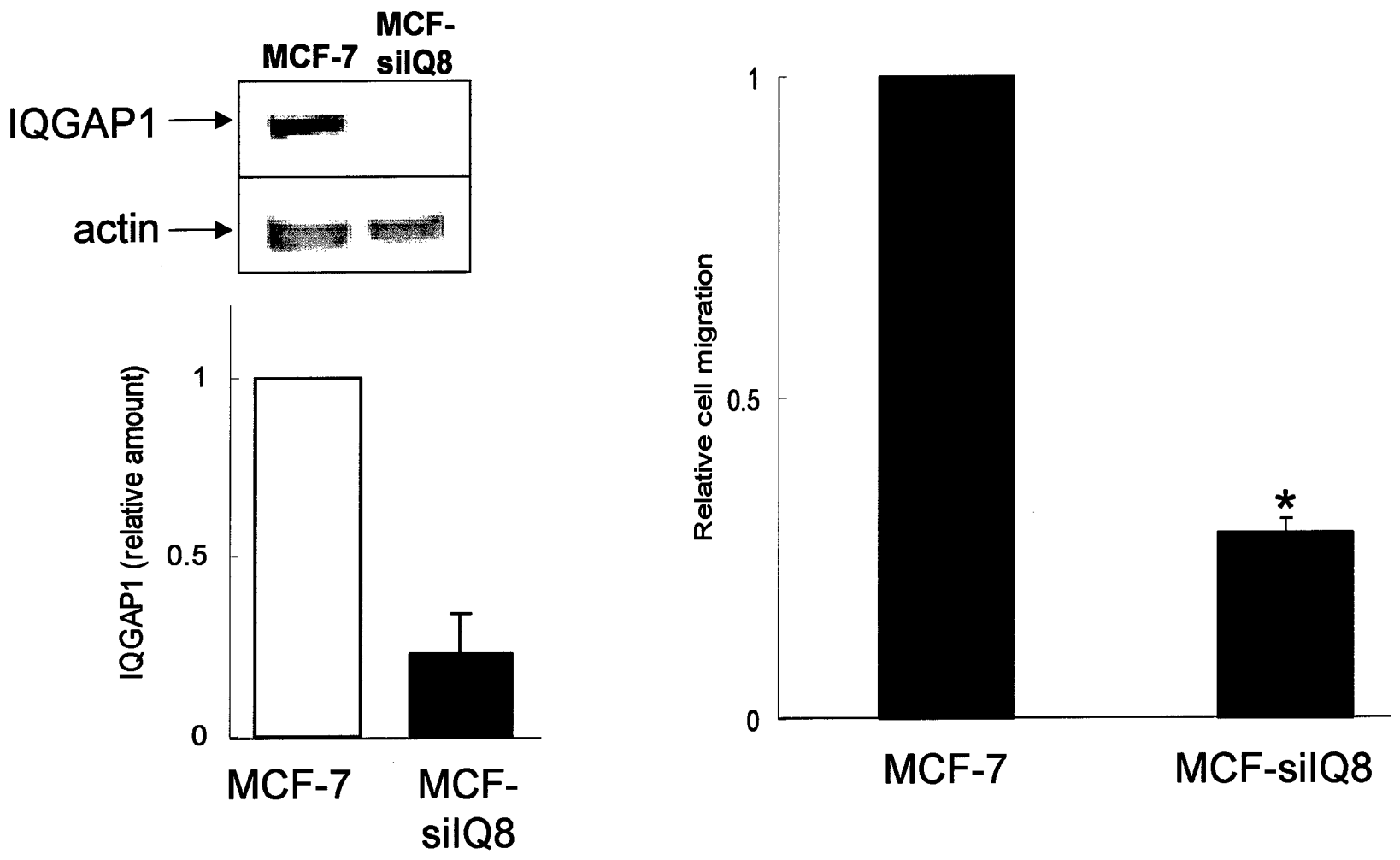

Fig. 2. Stable expression of siRNA for IQGAP1 reduces cell motility. *A*, equal amounts of protein lysates from MCF-7 and MCF-siIQ8 cells were analyzed by Western blotting with anti-IQGAP1 and anti-actin antibodies. IQGAP1 protein, quantified by densitometry and corrected for the amount of actin, is expressed relative to MCF-7 cells ($n = 2$). *B*, migration of MCF-7 and MCF-siIQ8 cells. Migration data, expressed relative to MCF-7 cells, represent the means ± S.E., $n = 3$. *, significantly different from MCF-7 cells ($p < 0.0001$).

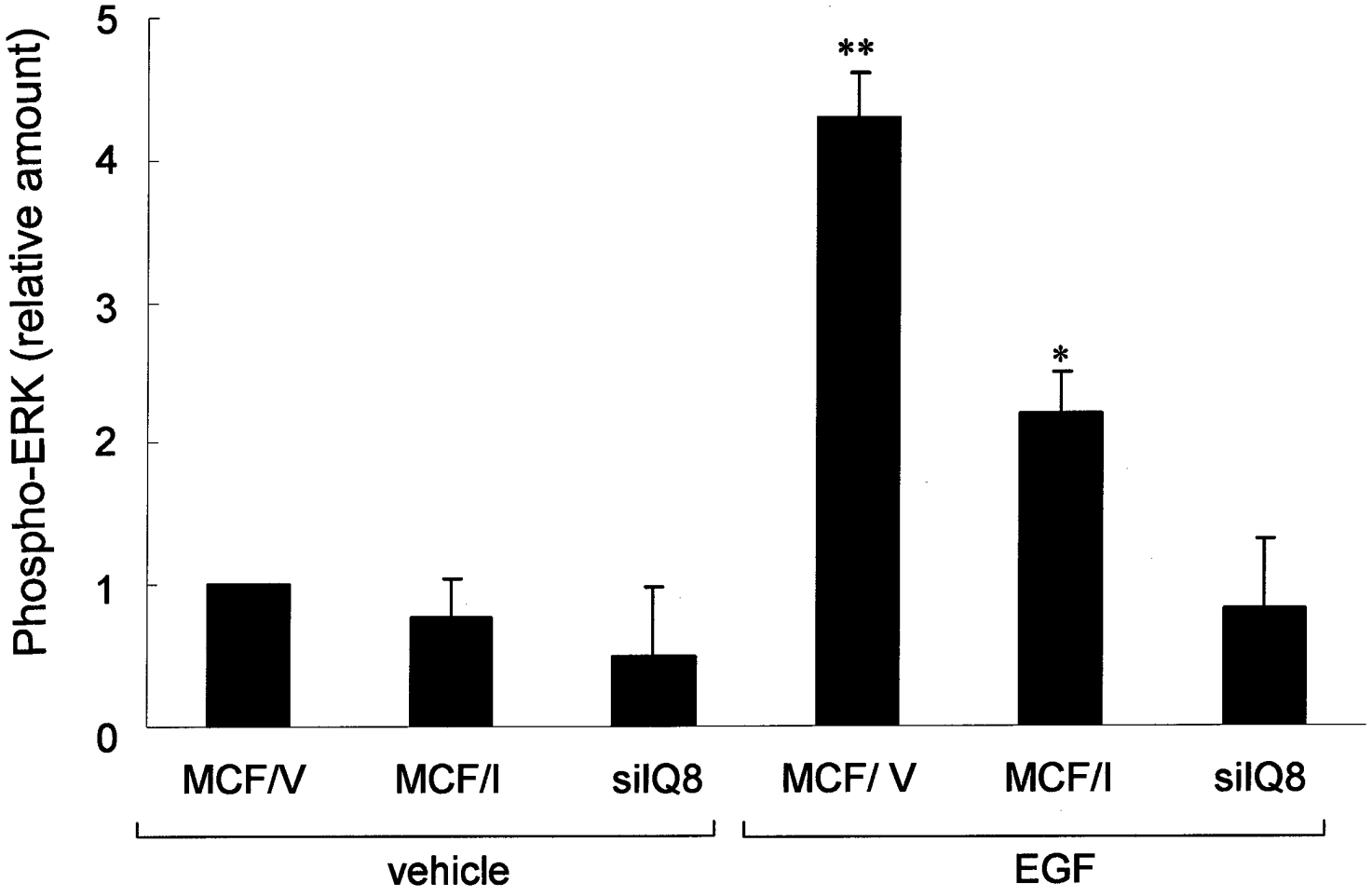

Fig. 3. IQGAP1 modulates EGF-stimulated ERK activity. ERK2 activity was examined in MCF-7 cells stably expressing either vector (MCF/V), IQGAP1 (MCF/I) or siRNA for IQGAP1 (siIQ8). Cells were starved of serum overnight and treated with vehicle or 100 ng/ml EGF for 10 min. The amount of phosphorylated ERK isoforms (Phospho-ERK) was quantified by densitometry and corrected for the amount of ERK2 in the corresponding lysate. Data, expressed relative to the amount of phospho-ERK in vehicle-treated MCF/V cells, represent the means ± S.E. (n=4). *, significantly different from vehicle-treated MCF/V cells ($p<0.01$). **, significantly different from vehicle-treated MCF/V cells ($p<0.001$).